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FILE 'HOME' ENTERED AT 17:59:49 ON 06 MAY 2002

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FILE 'MEDLINE' ENTERED AT 17:59:58 ON 06 MAY 2002

FILE 'BIOSIS' ENTERED AT 17:59:58 ON 06 MAY 2002
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=> fluorescence polarization
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=> s fluorescence polarization
L1 9673 FLUORESCENCE POLARIZATION

=> s l1 and (multiple or double or multiplex)
L2 506 L1 AND (MULTIPLE OR DOUBLE OR MULTIPLEX)

=> s l1 and multiplex
L3 1 L1 AND MULTIPLEX

=> d bib ab

L3 ANSWER 1 OF 1 MEDLINE
AN 2001113210 MEDLINE
DN 20517709 PubMed ID: 11062440
TI Wavelength-shifting molecular beacons.
AU Tyagi S; Marras S A; Kramer F R
CS Department of Molecular Genetics, Public Health Research Institute, 455 First Avenue, New York, NY 10016, USA.. sanjay@phri.nyu.edu
NC ES-10536 (NIEHS)
HL-43521 (NHLBI)
SO NATURE BIOTECHNOLOGY, (2000 Nov) 18 (11) 1191-6.
Journal code: CQ3. ISSN: 1087-0156.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200102
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010215
AB We describe wavelength-shifting molecular beacons, which are nucleic acid hybridization probes that fluoresce in a variety of different colors, yet are excited by a common monochromatic light source. The twin functions of absorption of energy from the excitation light and emission of that energy in the form of fluorescent light are assigned to two separate fluorophores in the same probe. These probes contain a harvester fluorophore that absorbs strongly in the wavelength range of the monochromatic light source, an emitter fluorophore of the desired emission color, and a nonfluorescent quencher. In the absence of complementary nucleic acid targets, the probes are dark, whereas in the presence of targets, they fluoresce-not in the emission range of the harvester fluorophore that absorbs the light, but rather in the emission range of the emitter fluorophore. This shift in emission spectrum is due to the transfer of the absorbed energy from the harvester fluorophore to the emitter fluorophore by fluorescence resonance energy transfer, and it only takes place in probes that are bound to targets. Wavelength-shifting molecular beacons are substantially brighter than conventional molecular beacons that contain a fluorophore that cannot efficiently absorb energy from the available monochromatic light source. We describe the spectral characteristics of wavelength-shifting molecular beacons, and we demonstrate how their use improves and simplifies **multiplex** genetic analyses.

=> s l2 and py<1999
L4 412 L2 AND PY<1999

=> d l2 1-10 bib ab

L2 ANSWER 1 OF 506 MEDLINE
AN 2002235842 IN-PROCESS
DN 21970096 PubMed ID: 11972350
TI Effect of primary and secondary structure of oligodeoxyribonucleotides on the fluorescent properties of conjugated dyes.
AU Nazarenko Irina; Pires Rick; Lowe Brian; Obaidy Mohamad; Rashtchian Ayoub
CS Invitrogen Corporation, 1620 Faraday Avenue, Carlsbad, CA 92008, USA.
SO NUCLEIC ACIDS RESEARCH, (2002 May 1) 30 (9) 2089-195.
Journal code: 0411011. ISSN: 1362-4962.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020426
Last Updated on STN: 20020426
AB We studied fluorescence intensity, polarization and lifetime of some commonly used fluorophores conjugated to oligodeoxyribonucleotides with different primary and secondary structures. We found that fluorescence intensity can increase or decrease upon hybridization of the labeled strand to its complement depending on the sequence and position of the fluorophore. Up to 10-fold quenching of the fluorescence upon hybridization was observed when the dye moiety was attached close to the 3' end and the 3'-terminal base was either dG or dC. No quenching upon hybridization was observed when the dye was positioned within the same sequence context but close to the 5' end. The presence of a dG overhang quenches the fluorescence less efficiently than a blunt end dG-dC or dC-dG base pair. When located internally in the **double** strand, the dG-dC base pair does not affect the fluorescence of the nearby dye. Guanosine in a single-stranded oligonucleotide quenches the fluorescence of nearby dye by <2-fold. Upon duplex formation, this quenching is eliminated and the fluorescence increases. This increase can only be detected when the fluorophore is located at least 6 nt from the terminal dG-dC base pair. The change of **fluorescence polarization** upon duplex formation inversely correlates with the change of intensity. Fluorescein conjugated to a single-stranded oligonucleotide or a duplex undergoes a bi-exponential decay with approximately 4 and approximately 1 ns lifetimes.

L2 ANSWER 2 OF 506 MEDLINE
AN 2002195898 IN-PROCESS
DN 21925650 PubMed ID: 11928759
TI Stains, labels and detection strategies for nucleic acids assays.
AU Kricka Larry J
CS Department of Pathology, University of Pennsylvania, Philadelphia 19104, USA.. kricka@mail.med.upenn.edu
SO ANNALS OF CLINICAL BIOCHEMISTRY, (2002 Mar) 39 (Pt 2) 114-29.
Journal code: 0324055. ISSN: 0004-5632.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020404
Last Updated on STN: 20020404
AB Selected developments and trends in stains, labels and strategies for detecting and measuring nucleic acids (DNA, RNA) and related molecules [e.g. oligo(deoxy)nucleotides, nucleic acid fragments and polymerase chain reaction products] are surveyed based on the literature in the final decade of the 20th century (1991-2000). During this period, important families of cyanine dyes were developed for sensitive detection of **double**-stranded DNA, single-stranded DNA, and oligo(deoxy)nucleotides in gels and in solution, and families of energy transfer primers were produced for DNA sequencing applications. The

continuing quest for improved labels for hybridization assays has produced a series of candidate labels including genes encoding enzymes, microparticles (e.g. quantum dots, nanocrystals, phosphors), and new examples of the fluorophore (e.g. cyanine dyes) and enzyme class of labels (e.g. firefly luciferase mutants). Label detection technologies for use in northern and southern blotting assays have focused on luminescent methods, particularly enhanced chemiluminescence for peroxidase labels and adamantyl 1,2-dioxetanes for alkaline phosphatase labels. Sets of labels have been selected to meet the demands of multicolour assays (e.g. four-colour sequencing and single nucleotide primer extension assays). Non-separation assay formats have emerged based on **fluorescence polarization**, fluorescence energy transfer (TaqMan, molecular beacons) and channelling principles. Microanalytical devices (microchips), high-throughput simultaneous test arrays (microarrays, gene chips), capillary electrophoretic analysis and dipstick devices have presented new challenges and requirements for nucleic acid detection, and fluorescent methods currently dominate in many of these applications.

L2 ANSWER 3 OF 506 MEDLINE
AN 2002188654 IN-PROCESS
DN 21895175 PubMed ID: 11897588
TI In vitro low-level resistance to azoles in Candida albicans is associated with changes in membrane lipid fluidity and asymmetry.
AU Kohli Avmeet; Smriti; Mukhopadhyay Kasturi; Rattan Ashok; Prasad Rajendra
CS Membrane Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi, India.
SO ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (2002 Apr) 46 (4) 1046-52. Journal code: 0315061. ISSN: 0066-4804.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020403
Last Updated on STN: 20020403
AB The present study tracks the development of low-level azole resistance in in vitro fluconazole-adapted strains of Candida albicans, which were obtained by serially passaging a fluconazole-susceptible dose-dependent strain, YO1-16 (fluconazole MIC, 16 microg ml(-1)) in increasing concentrations of fluconazole, resulting in strains YO1-32 (fluconazole MIC, 32 microg ml(-1)) and YO1-64 (MIC, 64 microg ml(-1)). We show that acquired resistance to fluconazole in this series of isolates is not a random process but is a gradually evolved complex phenomenon that involves **multiple** changes, which included the overexpression of ABC transporter genes, e.g., CDR1 and CDR2, and the azole target enzyme, ERG11. The sequential rise in fluconazole MICs in these isolates was also accompanied by cross-resistance to other azoles and terbinafine. Interestingly, fluorescent polarization measurements performed by using the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene revealed that there was a gradual increase in membrane fluidity of adapted strains. The increase in fluidity was reflected by observed change in membrane order, which was considerably decreased (decrease in **fluorescence polarization** values, P value) in the adapted strain (P value of 0.1 in YO1-64, compared to 0.19 in the YO1-16 strain). The phospholipid composition of the adapted strain was not significantly altered; however, ergosterol content was reduced in YO1-64 from that in the YO1-16 strain. The asymmetrical distribution of phosphatidylethanolamine (PE) between two monolayers of plasma membrane was also changed, with PE becoming more exposed to the outer monolayer in the YO1-64 strain. The results of the present study suggest for the first time that changes in the status of membrane lipid phase and asymmetry could contribute to azole resistance in C. albicans.

L2 ANSWER 4 OF 506 MEDLINE
AN 2002168927 MEDLINE
DN 21897998 PubMed ID: 11900537

TI 3'-5' exonuclease of Klenow fragment: role of amino acid residues within the single-stranded DNA binding region in exonucleolysis and duplex DNA melting.
AU Lam Wai-Chung; Thompson Elizabeth H Z; Potapova Olga; Sun Xiaojun Chen; Joyce Catherine M; Millar David P
CS Department of Molecular Biology, MB-19, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA.
NC GM28550 (NIGMS)
GM44060 (NIGMS)
SO BIOCHEMISTRY, (2002 Mar 26) 41 (12) 3943-51.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200204
ED Entered STN: 20020320
Last Updated on STN: 20020420
Entered Medline: 20020419
AB The mechanism of the 3'-5' exonuclease activity of the Klenow fragment of DNA polymerase I has been investigated with a combination of biochemical and spectroscopic techniques. Site-directed mutagenesis was used to make alanine substitutions of side chains that interact with the DNA substrate on the 5' side of the scissile phosphodiester bond. Kinetic parameters for 3'-5' exonuclease cleavage of single- and **double**-stranded DNA substrates were determined for each mutant protein in order to probe the role of the selected side chains in the exonuclease reaction. The results indicate that side chains that interact with the penultimate nucleotide (Q419, N420, and Y423) are important for anchoring the DNA substrate at the active site or ensuring proper geometry of the scissile phosphate. In contrast, side chains that interact with the third nucleotide from the DNA terminus (K422 and R455) do not participate directly in exonuclease cleavage of single-stranded DNA. Alanine substitutions of Q419, Y423, and R455 have markedly different effects on the cleavage of single- and **double**-stranded DNA, causing a much greater loss of activity in the case of a duplex substrate. Time-resolved fluorescence anisotropy decay measurements with a dansyl-labeled primer/template indicate that the Q419A, Y423A, and R455A mutations disrupted the ability of the Klenow fragment to melt duplex DNA and bind the frayed terminus at the exonuclease site. In contrast, the N420A mutation stabilized binding of a duplex terminus to the exonuclease site, suggesting that the N420 side chain facilitates the 3'-5' exonuclease reaction by introducing strain into the bound DNA substrate. Together, these results demonstrate that protein side chains that interact with the second or third nucleotides from the terminus can participate in both the chemical step of the exonuclease reaction, by anchoring the substrate in the active site or by ensuring proper geometry of the scissile phosphate, and in the prechemical steps of **double**-stranded DNA hydrolysis, by facilitating duplex melting.

L2 ANSWER 5 OF 506 MEDLINE
AN 2002064717 MEDLINE
DN 21650122 PubMed ID: 11790092
TI Determinants of DNA mismatch recognition within the polymerase domain of the Klenow fragment.
AU Thompson Elizabeth H Z; Bailey Michael F; van der Schans Edwin J C; Joyce Catherine M; Millar David P
CS Department of Molecular Biology, MB-19, The Scripps Research Institute, La Jolla, California 92037, USA.
NC GM28550 (NIGMS)
GM44060 (NIGMS)
SO BIOCHEMISTRY, (2002 Jan 22) 41 (3) 713-22.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)

LA English
FS Priority Journals
EM 200202
ED Entered STN: 20020125
Last Updated on STN: 20020214
Entered Medline: 20020213
AB The Klenow fragment of Escherichia coli DNA polymerase I catalyzes template-directed synthesis of DNA and uses a separate 3'-5' exonuclease activity to edit misincorporated bases. The polymerase and exonuclease activities are contained in separate structural domains. In this study, nine Klenow fragment derivatives containing mutations within the polymerase domain were examined for their interaction with model primer-template duplexes. The partitioning of the DNA primer terminus between the polymerase and 3'-5' exonuclease active sites of the mutant proteins was assessed by time-resolved fluorescence anisotropy, utilizing a dansyl fluorophore attached to the DNA. Mutation of N845 or R668 disrupted favorable interactions between the Klenow fragment and a duplex containing a matched terminal base pair but had little effect when the terminus was mismatched. Thus, N845 and R668 are required for recognition of correct terminal base pairs in the DNA substrate. Mutation of N675, R835, R836, or R841 resulted in tighter polymerase site binding of DNA, suggesting that the side chains of these residues induce strain in the DNA and/or protein backbone. A **double** mutant (N675A/R841A) showed an even greater polymerase site partitioning than was displayed by either single mutation, indicating that such strain is additive. In both groups of mutant proteins, the ability to discriminate between duplexes containing matched or mismatched base pairs was impaired. In contrast, mutation of K758 or Q849 had no effect on partitioning relative to wild type, regardless of DNA mismatch character. These results demonstrate that DNA mismatch recognition is dependent on specific amino acid residues within the polymerase domain and is not governed solely by thermodynamic differences between correct and mismatched base pairs. Moreover, this study suggests a mechanism whereby the Klenow fragment is able to recognize polymerase errors following a misincorporation event, leading to their eventual removal by the 3'-5' exonuclease activity.

L2 ANSWER 6 OF 506 MEDLINE
AN 2002053715 IN-PROCESS
DN 21637877 PubMed ID: 11779115
TI A **Fluorescence Polarization** Assay for the Identification of Inhibitors of the p53-DM2 Protein-Protein Interaction.
AU Knight Stephen M G; Umezawa Naoki; Lee Hee-Seung; Gellman Samuel H; Kay Brian K
CS Department of Pharmacology, University of Wisconsin-Madison, Madison, Wisconsin, 53706-1532.
SO ANALYTICAL BIOCHEMISTRY, (2002 Jan 15) 300 (2) 230-6.
Journal code: 0370535. ISSN: 0003-2697.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS IN-PROCESS; NONINDEXED; Priority Journals
ED Entered STN: 20020125
Last Updated on STN: 20020125
AB Improper function of the tumor suppressor protein p53 is a contributing factor in many human cancers. In normal cells, p53 acts to arrest the cell cycle in response to DNA damage or nucleotide depletion. One mechanism of regulating the amount of p53 in the cell is through the action of the **Double** Minute 2 protein, DM2 (also known as MDM2), which ubiquitinates p53 and targets it for proteosomal degradation. In a number of human cancers, the DM2 gene is amplified or overexpressed, leading to inadequate levels of p53 for cell cycle arrest or apoptosis. With the goal of restoring p53 function in cancers that overexpress DM2, we are developing inhibitors of the p53-DM2 protein-protein interaction that structurally mimic the N-terminal segment of p53 that binds to DM2. To assist this effort, we have devised a **fluorescence**

polarization assay that quantifies the interaction between the N-terminal regions of both proteins in 384-well microtiter plates. Using this assay, we have demonstrated that a peptide with a nonhydrolyzable beta-amino acid substitution binds DM2 with an affinity comparable to a p53 peptide that is composed of only alpha-amino acids. (c)2001 Elsevier Science.

L2 ANSWER 7 OF 506 MEDLINE
AN 2002050503 MEDLINE
DN 21633987 PubMed ID: 11772010
TI Equilibrium and stop-flow kinetic studies of fluorescently labeled DNA substrates with DNA repair proteins XPA and replication protein A.
AU Iakoucheva Lilia M; Walker Randall K; van Houten Ben; Ackerman Eric J
CS Pacific Northwest National Laboratory (PNNL), Molecular Biosciences Department, P.O. Box 999, Richland, Washington 99352, USA.
SO BIOCHEMISTRY, (2002 Jan 8) 41 (1) 131-43.
Journal code: 0370623. ISSN: 0006-2960.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200201
ED Entered STN: 20020125
Last Updated on STN: 20020130
Entered Medline: 20020129
AB Nucleotide excision repair (NER) is a crucial pathway in the maintenance of genome stability requiring at least two dozen proteins. XPA and RPA have essential roles in the damage recognition step of NER. To better understand the mechanism of their interactions with DNA, we utilized equilibrium and stop-flow kinetic approaches with fluorescently labeled oligonucleotides. Fluorescein is a bona fide NER lesion because a circular plasmid with a single defined fluorescein was repaired by efficient extracts from Xenopus oocyte nuclei. Single-stranded and **double** -stranded oligonucleotides 5'-labeled with fluorescein were used in the subsequent studies. Oligonucleotide fluorescence was quenched upon specific binding to full-length recombinant Xenopus XPA (xXPA) and/or human RPA. The binding was highly sensitive to the buffer conditions. Analysis of equilibrium binding data with ds DNA and xXPA revealed a single dissociation constant (K(d)) of 24.4 nM. Stopped-flow kinetic experiments were described by a first-order on-rate constant k(on) of 9.03 x 10(8) M(-1) s(-1) and k(off) of 26.1 s(-1). From the ratio of off-rate to on-rate, a calculated K(d) of 28.9 nM was obtained, revealing that the kinetic and equilibrium studies were consistent. The affinity of xXPA for ds undamaged DNA determined in our spectrofluorometry experiments was up to 3 orders of magnitude higher than previously reported values using different substrates, conditions, and assays [gel-shifts (EMSA), filter-binding, anisotropy, and surface plasmon resonance]. The same substrate DNA containing a 4-bp mismatch in the middle yielded a K(d) five times higher (158 nM), indicating weaker binding by xXPA. The differences in K(d) values for these two substrates were mainly attributable to the k(on), rather than k(off) rates. Fluorescence intensity changes upon interaction of xXPA with ss 50-mer were too low to calculate an accurate K(d). Although recombinant human RPA binding to the ds 50-mer was very weak (K(d) > 1 mM), stop-flow and equilibrium measurements to ss oligonucleotide yielded K(d) values of 96 and 20.3 nM, respectively, which correlated with previously reported values using gel mobility shift assays and a similarly sized poly-dT. Equilibrium and stop-flow measurements to the cognate and mismatched ds oligonucleotides using both xXPA and hRPA yielded a 2- to 3-fold increase in the K(d).

L2 ANSWER 8 OF 506 MEDLINE
AN 2002009886 MEDLINE
DN 21256625 PubMed ID: 11358153
TI Quantitative distance information on protein-DNA complexes determined in polyacrylamide gels by fluorescence resonance energy transfer.

AU Lorenz M; Diekmann S
CS Department of Molecular Biology, Institute for Molecular Biotechnology, Jena, Germany.
SO ELECTROPHORESIS, (2001 Apr) 22 (6) 990-8.
Journal code: 8204476. ISSN: 0173-0835.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011205
AB In polyacrylamide gels, we have quantitatively determined Forster transfer (fluorescense resonance energy transfer, FRET) between two fluorescent dyes attached to DNA in protein-DNA complexes. The donor-dye fluorescein labeled to DNA retains its free mobility in the polyacrylamide gel, however, its fluorescence properties change. The different quantum yield of fluorescein in the gel is found to be independent of the gel concentration and can thus be quantitatively taken into account by a reduced Forster distance R0 of 46 A compared to 50 A in solution. We have determined global structural properties of two proteins binding to **double**-labeled DNA using a novel gel-based fluorescence resonance energy transfer assay. In polyacrylamide gels we have analyzed the binding of integration host factor (IHF) and the high mobility group protein NHP6a to their substrate DNA. The measured Forster transfer efficiency allows us to deduce information on the overall shape and the DNA bending angle in the complex.

L2 ANSWER 9 OF 506 MEDLINE
AN 2002003457 MEDLINE
DN 21623655 PubMed ID: 11677244
TI Fluorescence-based analyses of the effects of full-length recombinant TAF130p on the interaction of TATA box-binding protein with TATA box DNA.
AU Banik U; Beechem J M; Klebanow E; Schroeder S; Weil P A
CS Department of Molecular Physiology and Biophysics, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232-0615, USA.
NC GM52461 (NIGMS)
GM5858 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Dec 28) 276 (52) 49100-9.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200201
ED Entered STN: 20020102
Last Updated on STN: 20020201
Entered Medline: 20020131
AB We have used a combination of fluorescence anisotropy spectroscopy and fluorescence-based native gel electrophoresis methods to examine the effects of the transcription factor IID-specific subunit TAF130p (TAF145p) upon the TATA box DNA binding properties of TATA box-binding protein (TBP). Purified full-length recombinant TAF130p decreases TBP-TATA DNA complex formation at equilibrium by competing directly with DNA for binding to TBP. Interestingly, we have found that full-length TAF130p is capable of binding **multiple** molecules of TBP with nanomolar binding affinity. The biological implications of these findings are discussed.

L2 ANSWER 10 OF 506 MEDLINE
AN 2001699996 MEDLINE
DN 21614987 PubMed ID: 11747549
TI Influence of an 8-oxoadenine lesion on the structural and dynamic features of a 30-mer DNA fragment with and without a mismatch.

AU Barone F; Cellai L; Giordano C; La Sala G; Mazzei F
CS Laboratorio di Fisica, Istituto Superiore di Sanita, Viale Regina Elena 299, I-00161 Rome, Italy.. barone@iss.it
SO INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, (2002 Jan) 78 (1) 9-16. Journal code: 8809243. ISSN: 0955-3002.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; S
EM 200202
ED Entered STN: 20011219
Last Updated on STN: 20020220
Entered Medline: 20020219
AB PURPOSE: To elucidate the influence of the oxidative lesion 7,8-dihydro-8-oxoadenine (8-oxoA) on the structural and dynamic features of a 30-mer DNA fragment, and to understand if differences occur when C is positioned opposite 8-oxoA instead of T. MATERIALS AND METHODS: Two 30-mer DNA oligomers with or without the 8-oxoA and two complementary oligomers with C or T base opposite the lesion site were synthesized and annealed. Duplexes named AT, A*T, AC and A*C were characterized by means of circular dichroism and UV denaturation measurements. gamma-Ray footprinting experiments were performed to give insight into their fine three-dimensional structure. Elastic torsional constants were derived by following the decay of the **fluorescence polarization** anisotropy (FPA) of the ethidium-DNA complexes measured by multifrequency-phase fluorometry. RESULTS: The introduction of one oxidative lesion in a 30-mer DNA oligomer with and without a mismatch did not cause relevant changes in their overall conformation and slightly modified their elastic properties. Small energetic differences were revealed by thermodynamic analysis in the sample bearing both the oxidative lesion and the mismatch. Minor variations in the cleavage pattern due to the hydroxyl radicals in the A*T sample were observed and present along the entire DNA fragment length. In the A*C sample, by contrast, there was a major modification in the cleavage pattern extending for about 11 bases around the lesion, especially towards the 5'-end. CONCLUSIONS: Differences in the fine structure and in the elastic properties between the A*T and A*C samples were observed, while their overall conformation was unchanged. The results are consistent with the hypothesis that the observed local changes of the **double** helix structure in A*C are due to the pairing of the oxidized adenine in a syn conformation with the cytosine.

=> d his

(FILE 'HOME' ENTERED AT 17:59:49 ON 06 MAY 2002)

FILE 'MEDLINE, BIOSIS' ENTERED AT 17:59:58 ON 06 MAY 2002
L1 9673 S FLUORESCENCE POLARIZATION
L2 506 S L1 AND (MULTIPLE OR DOUBLE OR MULTIPLEX)
L3 1 S L1 AND MULTIPLEX
L4 412 S L2 AND PY<1999

=> s l1 and (double label?)
L5 4 L1 AND (DOUBLE LABEL?)

=> d 1-4 bib ab

L5 ANSWER 1 OF 4 MEDLINE
AN 2002009886 MEDLINE
DN 21256625 PubMed ID: 11358153
TI Quantitative distance information on protein-DNA complexes determined in polyacrylamide gels by fluorescence resonance energy transfer.
AU Lorenz M; Diekmann S
CS Department of Molecular Biology, Institute for Molecular Biotechnology,

Jena, Germany.
SO ELECTROPHORESIS, (2001 Apr) 22 (6) 990-8.
Journal code: 8204476. ISSN: 0173-0835.
CY Germany: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200112
ED Entered STN: 20020121
Last Updated on STN: 20020121
Entered Medline: 20011205
AB In polyacrylamide gels, we have quantitatively determined Forster transfer (fluorescense resonance energy transfer, FRET) between two fluorescent dyes attached to DNA in protein-DNA complexes. The donor-dye fluorescein labeled to DNA retains its free mobility in the polyacrylamide gel, however, its fluorescence properties change. The different quantum yield of fluorescein in the gel is found to be independent of the gel concentration and can thus be quantitatively taken into account by a reduced Forster distance R0 of 46 A compared to 50 A in solution. We have determined global structural properties of two proteins binding to **double-labeled** DNA using a novel gel-based fluorescence resonance energy transfer assay. In polyacrylamide gels we have analyzed the binding of integration host factor (IHF) and the high mobility group protein NHP6a to their substrate DNA. The measured Forster transfer efficiency allows us to deduce information on the overall shape and the DNA bending angle in the complex.

L5 ANSWER 2 OF 4 MEDLINE
AN 94288275 MEDLINE
DN 94288275 PubMed ID: 7517105
TI Antibody-mediated fluorescence enhancement based on shifting the intramolecular dimer<-->monomer equilibrium of fluorescent dyes.
AU Wei A P; Blumenthal D K; Herron J N
CS Department of Pharmaceutics, University of Utah, Salt Lake City 84108.
NC AI 22898 (NIAID)
GM 08393 (NIGMS)
SO ANALYTICAL CHEMISTRY, (1994 May 1) 66 (9) 1500-6.
Journal code: 4NR; 0370536. ISSN: 0003-2700.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199407
ED Entered STN: 19940810
Last Updated on STN: 19970203
Entered Medline: 19940728
AB A novel concept is described for directly coupling fluorescence emission to protein-ligand binding. It is based on shifting the intramolecular monomer<-->dimer equilibrium of two fluorescent dyes linked by a short spacer. A 13-residue peptide, recognized by a monoclonal antibody against human chorionic gonadotrophin (hCG), was labeled with fluorescein (F) and tetramethylrhodamine (T) at its N- and C-terminus, respectively. Spectral evidence suggests that when the conjugate is free in solution, F and T exist as an intramolecular dimer. Fluorescence quenching of fluorescein and rhodamine is approximately 98% and approximately 90%, respectively, due to dimerization. When the **double-labeled** peptide is bound to anti-hCG, however, the rhodamine fluorescence increases by up to 7.8-fold, depending upon the excitation wavelength. This is attributed to the dissociation of intramolecular dimers brought about by conformational changes of the conjugate upon binding. Fluorescein fluorescence, on the other hand, was still quenched because of excited-state energy transfer and residual ground-state interactions. Antibody binding also resulted in a approximately 3.4-fold increase in fluorescence anisotropy of the peptide. These changes in intensity and anisotropy allow direct measurement of antigen-antibody binding with a

fluorescence plate reader or a polarization analyzer, without the need for separation steps and labeling antibodies. Because recent advances in peptide technology have allowed rapid and economical identification of antigen-mimicking peptides, the **double-labeled** peptide approach offers many opportunities for developing new diagnostic assays and screening new therapeutic drugs. It also has many potential applications to techniques involving recombinant antibodies, biosensors, cell sorting, and DNA probes.

L5 ANSWER 3 OF 4 MEDLINE
AN 92098618 MEDLINE
DN 92098618 PubMed ID: 1684584
TI Wild type and tailless CD8 display similar interaction with microfilaments during capping.
AU Andre P; Gabert J; Benoliel A M; Capo C; Boyer C; Schmitt-Verhulst A M; Malissen B; Bongrand P
CS Laboratoire d'Immunologie, Hopital de Sainte-Marguerite, Marseille, France.
SO JOURNAL OF CELL SCIENCE, (1991 Oct) 100 (Pt 2) 329-37.
Journal code: HNK; 0052457. ISSN: 0021-9533.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920223
Last Updated on STN: 19950206
Entered Medline: 19920203
AB We examined the influence of the intracytoplasmic region of CD8 alpha on capping and interaction with microfilaments. We used cell clones obtained by transfecting a CD4+ T-cell hybridoma with (a) T-cell receptor (TCR) alpha and beta chains from a cytolytic clone and (b) CD8 alpha genes that were either native or modified by extensive deletion of the intracytoplasmic region or replacement of the transmembrane and intracytoplasmic domains with those of a class I major histocompatibility complex gene (Letourneur et al. (1990). Proc. natn. Acad. Sci. U.S.A. 87, 2339-2343). Different cell surface structures were cross-linked with anti-T-cell receptor, anti-CD8 or anti-class I monoclonal antibodies and anti-immunoglobulin (Fab')2. **Double labeling** and quantitative image analysis were combined to monitor fluorescence anisotropy and correlation between different markers. Microfilaments displayed maximal polarization within two minutes. The correlation between these structures and surface markers was then maximal and started decreasing, whereas the redistribution of surface markers remained stable or continued. Furthermore, wild type and altered CD8 alpha exhibited similar ability to be capped and to induce co-capping of TCR and MHC (major histocompatibility complex) class I: the fraction of cell surface label redistributed into a localized cap ranged between 40% and 80%. Finally, cytochalasin D dramatically decreased CD8 capping in all tested clones. It is concluded that the transmembrane and/or intracellular domains of CD8 molecules are able to drive the extensive redistributions of membrane structures and cytoskeletal elements that are triggered by CD8 cross-linking.

L5 ANSWER 4 OF 4 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1990:459545 BIOSIS
DN BR39:94906
TI MANUAL OF LABORATORY IMMUNOLOGY SECOND EDITION.
AU MILLER L E; LUDKE H R; PEACOCK J E; TOMAR R H
CS STATE UNIV. NEW YORK HEALTH SCI. CENT. SYRACUSE, SYRACUSE, NEW YORK.
SO MILLER, L. E., H. R. LUDKE, J. E. PEACOCK AND R. H. TOMAR. MANUAL OF LABORATORY IMMUNOLOGY, SECOND EDITION. XIV+427P. LEA AND FEBIGER: MALVERN, PENNSYLVANIA, USA; KENT, ENGLAND, UK. ILLUS. PAPER. (1991) 0 (0), XIV+427P.
ISBN: 0-8121-1319-5.

DT Book
FS BR; OLD
LA English
AB This laboratory manual will meet the needs of a wide range of laboratory professionals including medical technologists, educators and students of medical technology, medical residents, and clinical pathologists. This text serves as a procedural manual of laboratory assays in humoral and cellular immunology, and a number of methods are described that are used in immunology laboratories. This second edition has an introductory chapter discussing the basic principles of the immune system. Chapters include an introduction to serologic methods, immunoglobulins, complement, phagocytes, infectious disease, serology, viral disorders, allergy, HLA antigens, and other topics of immunology. In this second edition new information on **double labeling** immunofluorescence tests for lymphocyte markers and flow cytometry, AIDS and HIV antibody testing, **fluorescence polarization**, hepatitis B, tests for autoantibodies and assays for tumor markers are included. This book contains many charts and graphs, references, and an index for easier access to information in the manual.

=> s fluorescence anisotropy
L6 3067 FLUORESCENCE ANISOTROPY

=> s l6 and (double label?)
L7 5 L6 AND (DOUBLE LABEL?)

=> d 1-5 bib ab

L7 ANSWER 1 OF 5 MEDLINE
AN 1998109359 MEDLINE
DN 98109359 PubMed ID: 9449305
TI Donor-donor energy migration for determining intramolecular distances in proteins: I. Application of a model to the latent plasminogen activator inhibitor-1 (PAI-1).
AU Karolin J; Fa M; Wilczynska M; Ny T; Johansson L B
CS Department of Physical Chemistry, Umea University, Sweden.
SO BIOPHYSICAL JOURNAL, (1998 Jan) 74 (1) 11-21.
Journal code: A5S; 0370626. ISSN: 0006-3495.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199804
ED Entered STN: 19980410
Last Updated on STN: 20000303
Entered Medline: 19980402
AB A new fluorescence spectroscopic method is presented for determining intramolecular and intermolecular distances in proteins and protein complexes, respectively. The method circumvents the general problem of achieving specific labeling with two different chromophoric molecules, as needed for the conventional donor-acceptor transfer experiments. For this, mutant forms of proteins that contain one or two unique cysteine residues can be constructed for specific labeling with one or two identical fluorescent probes, so-called donors (d). Fluorescence depolarization experiments on **double-labeled** Cys mutant monitor both reorientational motions of the d molecules, as well as the rate of intramolecular energy migration. In this report a model that accounts for these contributions to the **fluorescence anisotropy** is presented and experimentally tested. Mutants of a protease inhibitor, plasminogen activator inhibitor type-1 (PAI-1), containing one or two cysteine residues, were labeled with sulfhydryl specific derivatives of 4,4-difluoro-4-borata-3a-azonia-4a-aza-s-indacence (BODIPY). From the rate of energy migration, the intramolecular distance between the d groups was calculated by using the Forster mechanism and by accounting for the

influence of local anisotropic orientation of the d molecules. The calculated intramolecular distances were compared with those obtained from the crystal structure of PAI-1 in its latent form. To test the stability of parameters extracted from experiments, synthetic data were generated and reanalyzed.

L7 ANSWER 2 OF 5 MEDLINE
AN 94288275 MEDLINE
DN 94288275 PubMed ID: 7517105
TI Antibody-mediated fluorescence enhancement based on shifting the intramolecular dimer<-->monomer equilibrium of fluorescent dyes.
AU Wei A P; Blumenthal D K; Herron J N
CS Department of Pharmaceutics, University of Utah, Salt Lake City 84108.
NC AI 22898 (NIAID)
GM 08393 (NIGMS)
SO ANALYTICAL CHEMISTRY, (1994 May 1) 66 (9) 1500-6.
Journal code: 4NR; 0370536. ISSN: 0003-2700.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199407
ED Entered STN: 19940810
Last Updated on STN: 19970203
Entered Medline: 19940728
AB A novel concept is described for directly coupling fluorescence emission to protein-ligand binding. It is based on shifting the intramolecular monomer<-->dimer equilibrium of two fluorescent dyes linked by a short spacer. A 13-residue peptide, recognized by a monoclonal antibody against human chorionic gonadotrophin (hCG), was labeled with fluorescein (F) and tetramethylrhodamine (T) at its N- and C-terminus, respectively. Spectral evidence suggests that when the conjugate is free in solution, F and T exist as an intramolecular dimer. Fluorescence quenching of fluorescein and rhodamine is approximately 98% and approximately 90%, respectively, due to dimerization. When the **double-labeled** peptide is bound to anti-hCG, however, the rhodamine fluorescence increases by up to 7.8-fold, depending upon the excitation wavelength. This is attributed to the dissociation of intramolecular dimers brought about by conformational changes of the conjugate upon binding. Fluorescein fluorescence, on the other hand, was still quenched because of excited-state energy transfer and residual ground-state interactions. Antibody binding also resulted in a approximately 3.4-fold increase in **fluorescence anisotropy** of the peptide. These changes in intensity and anisotropy allow direct measurement of antigen-antibody binding with a fluorescence plate reader or a polarization analyzer, without the need for separation steps and labeling antibodies. Because recent advances in peptide technology have allowed rapid and economical identification of antigen-mimicking peptides, the **double-labeled** peptide approach offers many opportunities for developing new diagnostic assays and screening new therapeutic drugs. It also has many potential applications to techniques involving recombinant antibodies, biosensors, cell sorting, and DNA probes.

L7 ANSWER 3 OF 5 MEDLINE
AN 92098618 MEDLINE
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TI Wild type and tailless CD8 display similar interaction with microfilaments during capping.
AU Andre P; Gabert J; Benoliel A M; Capo C; Boyer C; Schmitt-Verhulst A M; Malissen B; Bongrand P
CS Laboratoire d'Immunologie, Hopital de Sainte-Marguerite, Marseille, France.
SO JOURNAL OF CELL SCIENCE, (1991 Oct) 100 (Pt 2) 329-37.
Journal code: HNK; 0052457. ISSN: 0021-9533.
CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199202
ED Entered STN: 19920223
Last Updated on STN: 19950206
Entered Medline: 19920203
AB We examined the influence of the intracytoplasmic region of CD8 alpha on capping and interaction with microfilaments. We used cell clones obtained by transfecting a CD4+ T-cell hybridoma with (a) T-cell receptor (TCR) alpha and beta chains from a cytolytic clone and (b) CD8 alpha genes that were either native or modified by extensive deletion of the intracytoplasmic region or replacement of the transmembrane and intracytoplasmic domains with those of a class I major histocompatibility complex gene (Letourneur et al. (1990). Proc. natn. Acad. Sci. U.S.A. 87, 2339-2343). Different cell surface structures were cross-linked with anti-T-cell receptor, anti-CD8 or anti-class I monoclonal antibodies and anti-immunoglobulin (Fab')2. **Double labeling** and quantitative image analysis were combined to monitor **fluorescence anisotropy** and correlation between different markers. Microfilaments displayed maximal polarization within two minutes. The correlation between these structures and surface markers was then maximal and started decreasing, whereas the redistribution of surface markers remained stable or continued. Furthermore, wild type and altered CD8 alpha exhibited similar ability to be capped and to induce co-capping of TCR and MHC (major histocompatibility complex) class I: the fraction of cell surface label redistributed into a localized cap ranged between 40% and 80%. Finally, cytochalasin D dramatically decreased CD8 capping in all tested clones. It is concluded that the transmembrane and/or intracellular domains of CD8 molecules are able to drive the extensive redistributions of membrane structures and cytoskeletal elements that are triggered by CD8 cross-linking.

L7 ANSWER 4 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1998:251358 BIOSIS
DN PREV199800251358
TI Donor-donor energy migration for determining intramolecular distances in proteins: I. Application of a model to the latent plasminogen activator inhibitor-1 (PAI-1.
AU Karolin, Jan; Fa, Ming; Wilczynska, Malgorzata; Ny, Tor; Johnsson, Lennart B.-A. (1)
CS (1) Dep. Phys. Chem., Umea Univ., S-901 87 Umea Sweden
SO Biophysical Journal, (Jan., 1998) Vol. 74, No. 1, pp. 11-21. ISSN: 0006-3495.
DT Article
LA English
AB A new fluorescence spectroscopic method is presented for determining intramolecular and intermolecular distances in proteins and protein complexes, respectively. The method circumvents the general problem of achieving specific labeling with two different chromophoric molecules, as needed for the conventional donor-acceptor transfer experiments. For this, mutant forms of proteins that contain one or two unique cysteine residues can be constructed for specific labeling with one or two identical fluorescent probes, so-called donors (d). Fluorescence depolarization experiments on **double-labeled** Cys mutant monitor both reorientational motions of the d molecules, as well as the rate of intramolecular energy migration. In this report a model that accounts for these contributions to the **fluorescence anisotropy** is presented and experimentally tested. Mutants of a protease inhibitor, plasminogen activator inhibitor type-1 (PAI-1), containing one or two cysteine residues, were labeled with sulfhydryl specific derivatives of 4,4-difluoro-4-borata-3a-azonia-4a-aza-s-indacence (BODIPY). From the rate of energy migration, the intramolecular distance between the d groups was calculated by using the Forster mechanism and by accounting for the influence of local anisotropic orientation of the d molecules. The

calculated intramolecular distances were compared with those obtained from the crystal structure of PAI-1 in its latent form. To test the stability of parameters extracted from experiments, synthetic data were generated and reanalyzed.

L7 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1992:23966 BIOSIS
DN BA93:13241
TI WILD TYPE AND TAILLESS CD8 DISPLAY SIMILAR INTERACTION WITH MICROFILAMENTS DURING CAPPING.
AU ANDRE P; GABERT J; BENOLIEL A M; CAPO C; BOYER C; SCHMITT-VERHULST A M; MALISSEN B; BONGRAND P
CS LABORATORIE D'IMMUNOLOGIE, HOSPITAL DE SAINTE-MARGUERITE, BP 29, 13277 MARSEILLE CEDEX 09, FRANCE.
SO J CELL SCI, (1991) 100 (2), 329-338.
CODEN: JNCSAI. ISSN: 0021-9533.
FS BA; OLD
LA English
AB We examined the influence of the intracytoplasmic region of CD8.alpha. on capping and interaction with microfilaments. We used cell clones obtained by transfecting a CD4+ T-cell hybridoma with (a) T-cell receptor (TCR) .alpha. and .beta. chains from a cytolytic clone and (b) CD.alpha. genes that were either native or modified by extensive deletion of the intracytoplasmic region or replacement of the transmembrane and intracytoplasmic domains with those of a class I major histocompatibility complex gene (Letourneur et al. (1990). Proc. natn. Acad. Sci. U.S.A. 87, 2339-2343). Different cell surface structures were cross-linked with anti-T-cell receptor, anti-CD8 or anti-class I monoclonal antibodies and anti-immunoglobulin (Fab')2. **Double labeling** and quantitative image analysis were combined to monitor **fluorescence anisotropy** and correlation between different markers. Microfilaments displayed maximal polarization within two minutes. The correlation between these structures and surface markers was then maximal and started decreasing, whereas the redistribution of surface markers remained stable or continued. Furthermore, wild type and altered CD8.alpha. exhibited similar ability to be capped and to induce co-capping of TCR and MHC (major histocompatibility complex) class I: the fraction of cell surface label redistributed into a localized cap ranged between 40% and 80%. Finally, cytochalasin D dramatically decreased CD8 capping in all tested clones. It is concluded that the transmembrane and/or intracellular domains of CD8 molecules are able to drive the extensive redistributions of membrane structures and cytoskeletal elements that are triggered by CD8 cross-linking.

```
=> d his

     (FILE 'HOME' ENTERED AT 17:59:49 ON 06 MAY 2002)

     FILE 'MEDLINE, BIOSIS' ENTERED AT 17:59:58 ON 06 MAY 2002
L1          9673 S FLUORESCENCE POLARIZATION
L2           506 S L1 AND (MULTIPLE OR DOUBLE OR MULTIPLEX)
L3             1 S L1 AND MULTIPLEX
L4           412 S L2 AND PY<1999
L5             4 S L1 AND (DOUBLE LABEL?)
L6          3067 S FLUORESCENCE ANISOTROPY
L7             5 S L6 AND (DOUBLE LABEL?)

=> s l6 and multiplex
L8             0 L6 AND MULTIPLEX

=>

---Logging off of STN---
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=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	23.16	23.37

STN INTERNATIONAL LOGOFF AT 18:13:23 ON 06 MAY 2002

Search Paper 8

WEST Search History

DATE: Monday, May 06, 2002

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L4	l1 same (multiple label$)	2	L4
L3	l1 same multiplex	0	L3
L2	L1 same (double label$)	1	L2
L1	fluorescence polarization	1420	L1

END OF SEARCH HISTORY

WEST

Your wildcard search against 2000 terms has yielded the results below

Search for additional matches among the next 2000 terms

Generate Collection | Print

Search Results - Record(s) 1 through 2 of 2 returned.

1. Document ID: US 20010046050 A1

L5: Entry 1 of 2 File: PGPB Nov 29, 2001

PGPUB-DOCUMENT-NUMBER: 20010046050
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010046050 A1

TITLE: Instantaneous dual band fluorescence detection systems

PUBLICATION-DATE: November 29, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hoyt, Clifford C.	Needham	MA	US	

US-CL-CURRENT: 356/417

ABSTRACT:

An apparatus for performing fluorescence detection of two or more biochemical probes and/or fluorescence measurement of fluorescence intensity at two or more spectral bands of light emitted from at least one sample spot is disclosed. The apparatus simultaneously directs emitted fluorescent light from multiple probes and/or at multiple spectral bands to different spots on a single pixelated detector.

L5: Entry 1 of 2 File: PGPB Nov 29, 2001

DOCUMENT-IDENTIFIER: US 20010046050 A1
TITLE: Instantaneous dual band fluorescence detection systems

Detail Description Paragraph (25):
[0065] The ability to obtain fluorescence polarization data in two spectral bands, with high precision in each, is very powerful. Precision is one of the central FIGS. of merit in fluorescence polarization measurements, and also in most spectral band ratios. When performing dual-probe fluorescence polarization measurements, the requirement for precision in both bands is paramount. This is because there is inevitably spectral cross-talk between bands; that is, each probe will emit to some degree into both bands. This places more extreme signal-to-noise demands on the instrument, compared to a single-probe measurement. One must unmix the spectral cross-talk, and determine what proportion of the signal in each band came from which probe, in order to assess the degree of fluorescence polarization in each probe. Techniques for this unmixing and data analysis are taught in co-pending patent application "Multiple Label Fluorescence Polarization Assay System and Method", by the same inventor, filed the same day as this, the contents of which are hereby incorporated in full and made a part of this application.

2. Document ID: US 20010033374 A1

L5: Entry 2 of 2 File: PGPB Oct 25, 2001

PGPUB-DOCUMENT-NUMBER: 20010033374
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20010033374 A1

TITLE: Multiple label fluorescence polarization assay system and method

PUBLICATION-DATE: October 25, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hoyt, Clifford C.	Needham	MA	US	

US-CL-CURRENT: 356/317; 250/458.1, 356/417

ABSTRACT:

A sample having a plurality of probe molecules is illuminated with at least one beam of excitation light that is linearly polarized along a first axis, thereby effecting fluorescence emission in a plurality of spectral bands. The intensity of a first component of fluorescence emission that is polarized along the first axis, as well as the intensity of a second component of fluorescence emission that is polarized along an orthogonal second axis, is measured for each of said plurality of spectral bands. These measurements are represented as a measurement vector M. Since each probe emits some limited amount of light in the characteristic band of another probe, this results in cross-talk between probes. The measurement vector is therefore corrected using an instrument response matrix A, which is generated by measuring the flux output of control samples which each have only a single probe species. A flux vector S is calculated according to S=A.sup.-1M, and the fluorescence polarization FP is calculated from the S values.

L5: Entry 2 of 2 File: PGPB Oct 25, 2001

DOCUMENT-IDENTIFIER: US 20010033374 A1
TITLE: Multiple label fluorescence polarization assay system and method

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Term	Documents
MULTIPLE.DWPI,EPAB,JPAB,USPT,PGPB.	940972
MULTIPLES.DWPI,EPAB,JPAB,USPT,PGPB.	30522
LABEL$	0
LABEL.DWPI,EPAB,JPAB,USPT,PGPB.	116110
LABELA.DWPI,EPAB,JPAB,USPT,PGPB.	3
LABELABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	22
LABELABITY.DWPI,EPAB,JPAB,USPT,PGPB.	1
LABELABLE.DWPI,EPAB,JPAB,USPT,PGPB.	210
LABELACCOUNT.DWPI,EPAB,JPAB,USPT,PGPB.	2
LABELACMPR.DWPI,EPAB,JPAB,USPT,PGPB.	6
LABELACTION.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L1 SAME (MULTIPLE LABEL$)).USPT,PGPB,JPAB,EPAB,DWPI.	2

There are more results than shown above. Click here to view the entire set.

Display Format: [ ] Change Format

Previous Page Next Page

WEST

Your wildcard search against 2000 terms has yielded the results below

Search for additional matches among the next 2000 terms

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Search Results - Record(s) 1 through 1 of 1 returned.

☐ 1. Document ID: US 6287770 B1

L2: Entry 1 of 1 File: USPT Sep 11, 2001

US-PAT-NO: 6287770
DOCUMENT-IDENTIFIER: US 6287770 B1

TITLE: Nucleic acid promoters

DATE-ISSUED: September 11, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Weston; Anthony	Northolt			GBX
Assenberg; Rene	Banbury			GBX
Marsh; Peter	Leamington Spa			GBX
Mock; Graham A	Thame			GBX
Ray; Trevor D.	Abindgon			GBX
Wharam; Susan D	Coventry			GBX
Cardy; Donald L. N.	Aston Le Walls			GBX

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2, 536/22.1, 536/23.1, 536/24.1, 536/24.3, 536/24.33

ABSTRACT:

Disclosed is a method of detecting the presence of a nucleic acid target sequence of interest, the method comprising the steps of:

(a) adding first and second nucleic acid probes to a sample comprising the sequence of interest, so as to form a complex comprising three strands of nucleic acid, wherein the first probe comprises the full length sequence of a first strand of a double stranded promoter, the target sequence comprises an end part of a second strand of the double stranded promoter which is complementary to a part of the first strand, and the second probe comprises the rest of the second strand of the double stranded promoter which is complementary to a part of the first strand, such that a functional promoter is formed when the first probe is hybridized to both the target sequence and to the second probe;

(b) adding a polymerase which recognizes the promoter, so as to cause the de novo synthesis of nucleic acid from the promoter present in the complex; and

(c) detecting directly or indirectly the de novo synthesized nucleic acid. Also disclosed is the complex formed in performance of the method defined above, and a kit for performing the method defined above.

22 Claims, 16 Drawing figures

Exemplary Claim Number: 1
Number of Drawing Sheets: 16
L2: Entry 1 of 1 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6287770 B1
TITLE: Nucleic acid promoters

Detailed Description Paragraph Right (101):
5 .mu.l aliquots of sample or 5 .mu.l of a suitable dilution of the treated assay sample are added to 100 .mu.l buffer (50 mM Tris-HCl pH7.5, 20 mM MgCl.sub.2, 10% ethanol), followed by 10 pmol probe 3. This double-labelled RNA (5'-Tamra, 3'-Fam) is the ribozyme substrate. The RNA product of the 21/2 way junction (formed in the presence of specific target) is designed to be the corresponding "hammerhead" ribozyme. Probe 3 therefore anneals to the RNA product, creating a functional ribozyme. Ribozyme cleavage of the substrate, which results in the removal of the quencher from the fluorophore, can be monitored by Fluorescence Resonance Energy Transfer (Tamra excitation at 546 nm, emission at 579 nm). Alternatively, substrate cleavage could be measured by a decrease in fluorescence polarisation. Since substrate turnover is possible, a level of amplification may be achieved during the detection process.

Generate Collection | Print

Term	Documents
DOUBLE.DWPI,EPAB,JPAB,USPT,PGPB.	798550
DOUBLES.DWPI,EPAB,JPAB,USPT,PGPB.	16429
LABEL$	0
LABEL.DWPI,EPAB,JPAB,USPT,PGPB.	116110
LABELA.DWPI,EPAB,JPAB,USPT,PGPB.	3
LABELABILITY.DWPI,EPAB,JPAB,USPT,PGPB.	22
LABELABITY.DWPI,EPAB,JPAB,USPT,PGPB.	1
LABELABLE.DWPI,EPAB,JPAB,USPT,PGPB.	210
LABELACCOUNT.DWPI,EPAB,JPAB,USPT,PGPB.	2
LABELACMPR.DWPI,EPAB,JPAB,USPT,PGPB.	6
LABELACTION.DWPI,EPAB,JPAB,USPT,PGPB.	1
(L1 SAME (DOUBLE LABEL$)).USPT,PGPB,JPAB,EPAB,DWPI.	1

There are more results than shown above. Click here to view the entire set.

Display Format: REV, K | Change Format